3. Micro-burners;
4. Ostwald pipettes of 1 and 2 cc. capacity;
5. Condensers of small size.[1]

The fume absorption apparatus consists of two parts: (1) a piece of straight glass tubing with side arms, (2) the fume absorber proper. This latter is a 25-cc. pipette, one end of which is invaginated into the bulb, the other bent midway at a little more than right angles. The invaginated end sits into the neck of the digestion flask, while the other end fits into a side arm of the glass tubing. The latter, in turn, connects to the suction pump, by which the fumes are drawn off. Both the Kjeldahl flasks and the tubing are supported in the manner illustrated in pl. 7.

In the Folin modification, Jena test-tubes (200×25 mm.) are used in digestion. In this laboratory the small Kjeldahl flask has been found to be better adapted to plant material, because of the relatively high percentage of carbohydrates present, and the tendency of these to froth.

The material, if in solution, is added to the digestion flask by means of a calibrated Ostwald pipette; if in solid form, as plant organs or sections, it is carefully dried and weighed. The quantity of material taken for digestion must be determined by a preliminary rough analysis, since the method is best adapted for amounts of nitrogen between .5 and 5 mg. One cc. (more if needed) of chemically pure sulphuric acid (conc.) is added to the material to be digested, the amount depending upon the quantity of carbon-containing compounds present, then 1 gram of potassium sulphate and a drop of 5 per cent copper sulphate. The contents are heated slowly until frothing is over, after which a hotter flame may be employed. Sometimes it is necessary to add some solid fragment to prevent bumping, a bit of unglazed porcelain being especially good. Small mica chimneys can be obtained to protect the flames from air currents, but lacking these bottomless beakers may be used.

Upon completion of digestion the contents of the flask are permitted to cool somewhat (the liquid must not become

[1] The condensers can be made in the laboratory from glass tubing.

solid), then 50 cc. of ammonia-free water carefully added. Folin removed the nitrogen by adding saturated NaOH to alkalinity, then forcing the ammonia over into standard acid with a vigorous air current. While the method is excellent with the small amount of water used in the Jena tube, it does not give good results with the Kjeldahl flask and the larger amount of water used there. Distillation is more efficient.

Small condenser tubes were made in the laboratory from glass tubing, the outer jacket measuring 40×2 cm., and the inner 5 mm. in diameter. The lower end of this latter, where it dipped into the collection acid, was fitted with a larger tube 14 mm. in diameter—this to prevent back-flow of the acid; to the upper end of the inner tube was attached a safety trap made from a 10-cc. pipette, which, in turn, fitted into the Kjeldahl flask by means of a two-hole rubber stopper. Through the second hole of this was inserted a small piece of glass tubing closed at the upper end with a bit of rubber tubing and a pinch clamp, thus making it possible to add the alkali after the apparatus had been connected up for distillation. The distillation is carried on in the usual way. It is commonly necessary to add a pinch of zinc dust to the distilling mixture to prevent bumping, while a few drops of liquid paraffin will keep down a tendency to froth. The ammonia is collected in N/20 acid and titrated against alkali of the same strength. Alizarin red (Alizarin sulfonsäure Natrium, Merck) in .1 per cent aqueous solution gave best satisfaction as an indicator.

Folin has chiefly employed the colorimeter for the actual determination of nitrogen. The method has its distinct advantages, especially if the precautions indicated by Folin are observed in Nesslerizing. In the absence of a colorimeter, however, and because excellent results were always obtained in our work by titration, the latter method has been retained.

The following tables show how the results obtained with the "micro"-method approximate very closely those gotten with larger amounts of material in the original Kjeldahl. The illustration is that of an ordinary laboratory experiment

showing some of the steps involved in the enzymic hydrolysis of albumin.

Papain (.1 gm.) was added to 200 cc. 2.5 per cent albumin solution, alkalinity reduced to N/250, then incubated at 40°C. for two hours. Portions were removed and tested both with the "micro"- and the "macro"-Kjeldahl for proteolytic change. One cc. was digested with the former, 15 cc. were used with the latter.

TABLE I

NITROGEN DETERMINATIONS IN THE HYDROLYSIS OF ALBUMIN

Nitrogen fractions	Micro-Kjeldahl				Macro-Kjeldahl	
	Albumin +enzyme	Albumin +water	Albumin +enzyme	Albumin +water	Albumin +enzyme	Albumin +water
	in 1 cc.		calc. to 15 cc.		in 15 cc.	
	mg.	mg.	mg.	mg.	mg.	mg.
Total nitrogen....	3.80	3.795	57.00	56.925	57.675	57.500
Coagulable nitrogen.....	.913	3.145	13.695	47.175	14.125	48.225
Phosphotungstate ppt.....	1.397	.472	20.955	7.080	21.275	6.925
Amino acids and NH_4.....	1.490	.177	22.35	2.655	22.5025	2.785

Further comparison is made in the recovery of nitrogen from a carefully prepared solution of $(NH_4)_2SO_4$. As before, 1 cc. of solution was used with the "micro"- and 15 cc. with the "macro"-Kjeldahl.

TABLE II

THE RECOVERY OF NITROGEN FROM A SOLUTION OF $(NH_4)_2SO_4$ CONTAINING 3.217 MGS. PER CC.

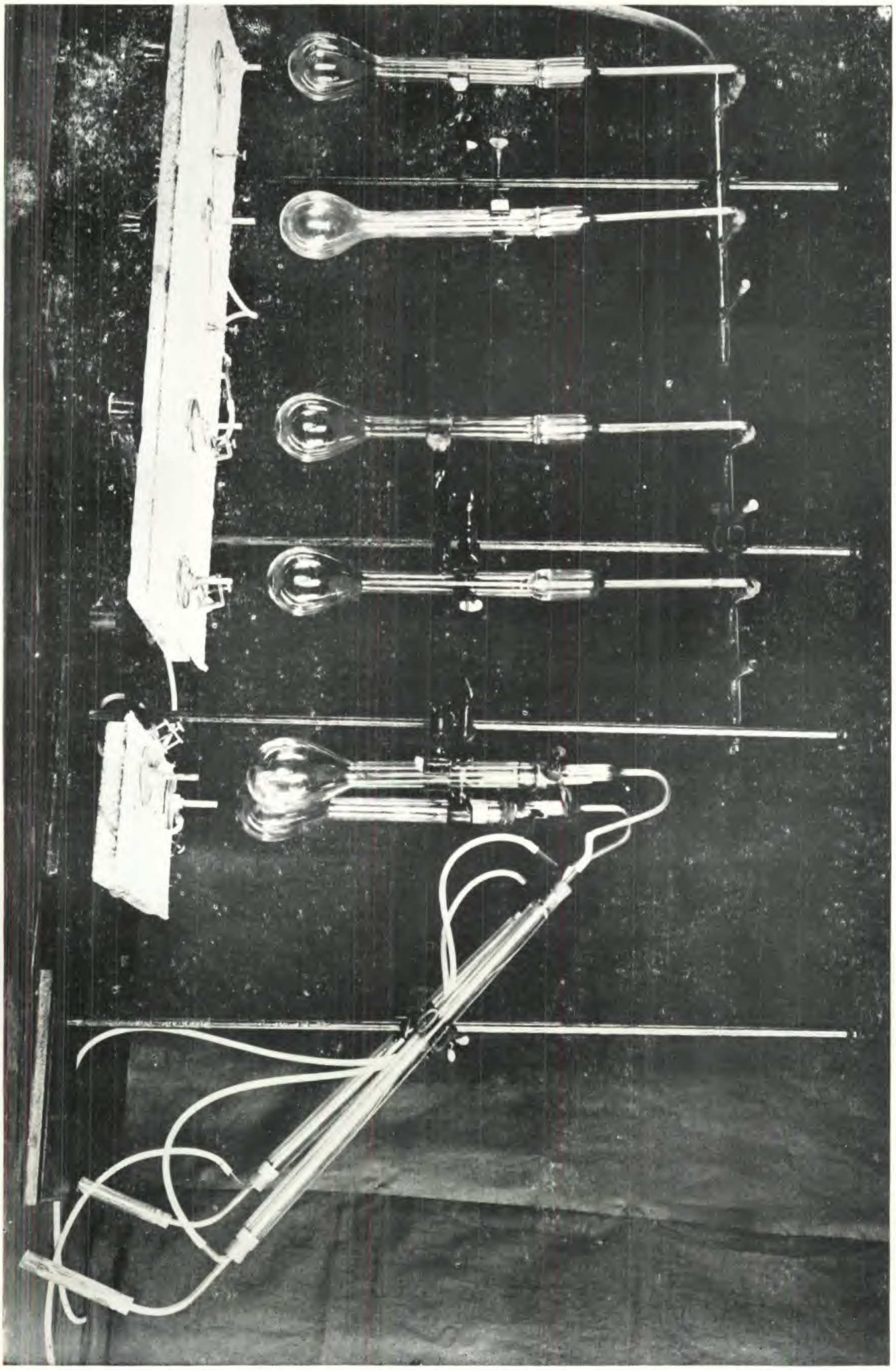
Exp. no.	Micro-Kjeldahl, 1 cc. solution used		Macro-Kjeldahl 15 cc. solution used
	Found	Calculated for 15 cc.	Found
	mg.	mg.	mg.
1	3.13	46.95	47.595
2	3.12	46.80	48.125
3	3.17	47.55	46.925
4	3.185	47.775	45.400
5	3.192	47.880	47.3925
Theoretical	3.217	48.255	48.255

Graduate Laboratory, Missouri Botanical Garden.

EXPLANATION OF PLATE

PLATE 7

Folin's micro-Kjeldahl apparatus for the determination of nitrogen.



DAVIS—FOLIN MICRO-KJELDAHL APPARATUS

STUDIES IN THE PHYSIOLOGY OF THE FUNGI[1]

I. Nitrogen Fixation

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Introduction and Critical Review of Literature

The problem of the fixation of free (atmospheric) or molecular nitrogen by the fungi has received attention at the hands of no small number of investigators, yet a careful study of the literature is sufficient to indicate that much further work —with the strictest regards for accurate methods—will be required before the problem is satisfactorily solved. For reasons developed later in this paper, we have felt the desirability of continuing, under different conditions, the investigations begun by one of us some years ago.

At the present time there can be no doubt entertained, of course, as to the capacity of the legume tubercle bacteria (*Bacillus radicicola* vars.) and certain soil forms (notably *Azotobacter spp.* and *Clostridium Pasteurianum*) to fix nitrogen. Here the amounts of nitrogen-increase in relatively small cultures under favorable conditions are so far above any regular experimental errors, and so consistently reported by careful workers, that the simple question of whether or not there is fixation is eliminated. On the other hand, there is much contradictory evidence as to the fact of nitrogen fixation by other bacteria and by the fungi, especially by the moulds and

[1] Note.—About half a dozen investigations are already in progress dealing with the physiology of the fungi, and it is proposed to give considerable attention to this phase of physiology during the next few years. The investigations would include certain aspects of nutrition and enzyme action, growth relations—especially the effects of environmental factors—and various phases of the general phenomenon of parasitism. On account of the continuity or relationship of many of the problems, it has seemed well to group these topics under the general title "Studies in the Physiology of the Fungi," of which the present article is No. 1. —B. M. Duggar.